29. The V_H and V_L polypeptides of any claims 22-27 wherein said V_H and V_L polypeptides comprise a whole antibody.

REMARKS

II. Preliminary Matters

Claims 16-21 have been cancelled without prejudice to the Applicants' right to pursue the subject matters of those claims in duly filed continuation application. The cancellation of claims 16-21 does not indicate acquiescence to the rejection but are rather being cancelled to expedite prosecution of the newly added claims.

New independent claim 22 recites subject matter of cancelled claim 16 but is written in independent form with additional amendments.

Claim 23 depends from claim 22.

New Claim 24 recites subject matter of cancelled claim 18 but is written in independent form with additional amendments.

New claim 25 depends from claim 24.

New claim 26 recites subject matter of cancelled claim 21 but is written in independent form with additional amendments.

New claim 27 depends from claim 26.

New claims 28 and 29 depend from claims 22-27.

III. Patentability Arguments

A. The Rejections Under 35 U.S.C. § 112, first paragraph, Should Be Withdrawn

Claims 16-21 cancelled and replaced as described above, were rejected for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one of ordinary skill in the art that the inventor(s) at the time the invention was filed had possession of the claimed invention.

On pages 2 and 3 of the Office Action, the Examiner characterized the claimed invention as being drawn to "a product," *i.e.*, a V_L or V_H polypeptide made by a particular method, and more narrowly, to a V_L or V_H polypeptide which is 'capable of having' catalytic activity.

The Examiner then digressed into a discussion of antibody structure-function relationships and how the structure-function relationship determines the specificity of an antibody. The Examiner went on to say how that structure-function relationship is and was insufficiently understood in the art to permit the structure of the antibody polypeptide to be known a priori based solely on its binding specificity. Further, the Examiner stated that the genus of possible antibody sequences is immense, and although a number of antibody polypeptide sequences is known in the art, the inability of the art to develop a theory which relate structure and function in antibodies renders such a sample of sequences inadequate to serve as a representative sample. The Examiner thus concluded that it would not have been apparent to one of skill in the art that the Applicants were in possession of the full scope claimed of the invention at the time of filing.

The Applicants respectfully traverse the rejection and request reconsideration in view of the following.

The present invention is concerned with a synthetic method of producing a diverse genetic library which is representative of the immune repertoire of an animal. More specifically, the present invention provides a method to clone a large number of different antibody variable region genes (i.e., V_L and V_H) for expression of these genes in expression vectors. The inventors described and used a set of DNA primers which are capable of hybridizing to a much larger number of antibody genes allowing amplification of these genes using PCR which results in a genetically diverse population of V_H or V_L coding sequences.

The Applicants have demonstrated generally that the methods taught give rise to a diversity of antibody-encoding sequences, which can be expressed in recombinant host cells. Antigens may be screened against the resulting library, and V_H and V_L sequences which bind an antigen of interest may be selected.

In principle, this is analogous to the traditional production of monoclonal antibodies by injecting an animal with an antigen, preparing a panel of hybridoma cells from the spleen cells of an animal, and selecting a hybridoma which is capable of binding the desired antigen. The skilled person is able to perform this without the knowledge of the detailed structure of the antibody-antigen interaction. In fact, for most skilled people, this is probably of little or no interest as long as the antibody binds the target antigen.

NO - STRUCTU CONSTRAINTS A UNITY HIGHER FOR FUNCTIONAL CATALYTIC Ab's

A particular class of target antigens of interest are those which are transition state analogues. These analogues may be used in the production of catalytic antibodies, as discussed in the literature cited in the present application at page 2 lines 26-32.

The Applicants have demonstrated that a library produced by the method contains antibodies that bind a particular transition state hapten (NPN) (See, *e.g.,* Figure 13 described in detail at page 10, lines 9-18, and Example 18, particularly 18C and pages 84-85 of the specification) which were subsequently shown to have the predicted catalytic activity. (Sastry *et al.*, *Catalytic Antibodies*, 1991, Ciba Foundation Symposium, 159, p. 145-155, attached hereto as Appendix B). The applicants were able to accomplish this without the detailed structure-function information discussed that the Examiner alleges would be needed to practice the invention as presently claimed and no further information would be required to practice the full scope of the invention.

YES, AND YOU HAD NO IDEA WHAT SEQ. OF AMINO ACID WOULD BE FOUND.

Indeed, the method of the present invention eliminates any need for the kind of structure-function information discussed by the Examiner other than the fact that the polypeptides are V_H and V_L polypeptides which are provided by the methods described in the specification. If in fact such information becomes desirable, one could obtain an antibody which binds to a given target and has a catalytic activity using the methods of the present invention and then analyze the structure of the antibody so obtained to ascertain any structure-function relationship. However, such information is demonstrably unnecessary to practice the presently claimed invention.

NO, STATUTY REQUIRES IT.

PRACTICE? NOT ENABLEMENT REJ.

In view of the above, the applicants respectfully request the rejection under 35 U.S.C. §112 first paragraph be withdrawn.

B. The Rejections Under 35 U.S.C. § 102(e) Should Be Withdrawn

Claims 16, 18 and 20 (see Amendment above) are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Huston (U.S. Patent No. 5,132,405, "Huston") and Sastry, *et al.*, published August 1989. The Applicants respectfully request reconsideration and request that the rejections be withdrawn for the following reasons.

The Examiner has characterized the instant claims as being drawn to V_H or V_L polypeptides. However, as amended, the claims recite V_H and V_L polypeptides which in combination have catalytic activity. However, Huston fails to teach catalytic V_H-V_L polypeptides as presently claimed and thus cannot properly anticipate the claims. On that basis, the applicants request that the rejections under 35 U.S.C. 102(e) be withdrawn.

The Examiner has also rejected Claims 16, 18 and 20 under 35 U.S.C. § 102(e) as allegedly being anticipated by Sastry, *et al.* These rejections should be withdrawn for at least two reasons. First, in order to qualify as a reference under 35 U.S.C. § 102(e), the reference

must be a U.S. patent. Sastry, *et al.*, is not a U.S. patent and therefore cannot anticipate the present claims under 35 U.S.C. § 102(e).

Second, Sastry, *et al.*, is not prior art with respect to the present application because it was published in August 1989, nearly three months after the priority date of the present invention and thus cannot anticipate the present invention.

In view of the foregoing, the applicants respectfully submit that the rejections under 35 U.S.C. § 102(e) should be withdrawn and withdrawal is requested.

C. The Rejections Under 35 U.S.C. § 112 Second Paragraph, Should Be Withdrawn

The Examiner has rejected claims 17, 19, 21 as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

The crux of the rejection lies in the use of the phrase "capable of having a catalytic activity" which the Examiner believes to be unclear.

The applicants respectfully submit that the rejection is moot in view of the present claims which recite "...V_H and V_L polypeptides **having** in combination with one another catalytic activity..." (emphasis added). The applicants submit that the phrase is clear, and therefore that the rejection is moot and should be withdrawn.

Conclusion

The Applicants submit that claims 22-24 are in condition for allowance and early notification thereof is solicited. The Commissioner is hereby authorized to charge any additional fees which may be required in the Application to Deposit Account No. 54-1214.

Respectfully submitted,

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By: _____
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APPENDIX A

22. A V_H and V_L polypeptide having in combination with one another a catalytic activity isolated by the method comprising the steps of:

(a) synthesizing a V_H and a V_L-coding gene library containing a plurality of different V_H and V_L -coding DNA sequences by a method comprising the steps of:

(i) preparing a first polynucleotide containing composition, wherein at least a portion of the polynucleotides in said composition comprise a plurality of different V_H-coding sequences;

(ii) preparing a second polynucleotide containing composition, wherein at least a portion of the polynucleotides in said composition comprise a plurality of different V_L-coding sequences;

(iii) amplifying a plurality of V_H and V_L-coding sequences in said respective polynucleotide containing compositions;

(iv) joining in operable combination, V_H and V_L-coding sequences from said V_H and V_L-coding gene library with expression vectors so as to be able to express a V_H and V_L-coding sequence from each vector, whereby a diverse library is formed;

(b) selecting and isolating from said diverse library at least one expression vector capable of producing V_H and V_L polypeptide having in combination with one another catalytic activity;

(c) transforming a host cell with said expression vector; and

(d) isolating a V_H and V_L polypeptide encoded by said vector from said host cell.

23. The V_H and V_L polypeptide of claim 22 wherein said V_H and V_L coding sequences from said V_H and V_L coding library are joined with separate expression vectors.

24. A V_H and V_L polypeptide having in combination with one another a catalytic activity isolated by a method comprising the steps of:

(a) preparing a first polynucleotide containing composition, wherein a portion of the polynucleotides in said composition comprise a plurality of different V_H-coding sequences;

(b) preparing a second polynucleotide containing composition, wherein a portion of the polynucleotides in said composition comprise a plurality of different V_L-coding sequences;

(c) amplifying a plurality of V_H and V_L-coding sequences from said first and said second polynucleotide containing compositions, respectively, by a method of amplification comprising the step of adding primers capable of hybridizing upstream and downstream from a plurality of said V_H coding sequences and adding primers capable of hybridizing upstream and downstream from a plurality of said V_L coding sequences, under conditions permitting hybridization to occur, whereby a plurality of different amplified V_H and a plurality of different amplified V_L coding sequences are produced;

(d) joining in operable combination, said amplified V_H and V_L-coding sequences with expression vectors so as to be able to express a V_H and V_L-coding sequence from each vector, whereby a diverse library is formed;

(e) selecting and isolating from said diverse library at least one expression vector capable of producing a V_H and V_L polypeptide which in combination with one another have said catalytic activity,

(f) transforming a host cell with said expression vector; and

(g) isolating a V_H and V_L polypeptide encoded by said vector from said host cell.

25. The V_H and V_L polypeptide of claim 24 wherein said amplified V_H and said amplified V_L coding sequences are joined with separate expression vectors.

26. A V_H and V_L polypeptide having in combination with one another a catalytic activity isolated by the method comprising the steps of:

(a) producing a V_H and V_L-coding genetic library, by a method comprising the steps of:

(i) adding a first primer, wherein said first primer is capable of hybridizing to a first conserved nucleotide sequence substantially adjacent to a plurality of V_H-coding regions, and said coding sequences are present in a polynucleotide containing composition that comprises a plurality of different V_H and V_L coding sequences;

(ii) adding a second primer to said polynucleotide containing composition, wherein said second primer is capable of hybridizing to a second

conserved nucleotide sequence substantially adjacent to a plurality of different V_H-coding regions;

(iii) adding a third primer, wherein said third primer is capable of hybridizing to a third conserved nucleotide sequence substantially adjacent to a plurality of V_L-coding regions;

(iv) adding a fourth primer to said polynucleotide containing composition, wherein said fourth primer is capable of hybridizing to a fourth conserved nucleotide sequence substantially adjacent to a plurality V_L-coding regions;

(v) amplifying said V_H coding sequences and said V_L coding sequences;

(b) joining in operable combination said amplified V_H and V_L-coding sequences with expression vectors so as to be able to express V_H and V_L-coding sequence from said vectors, whereby a diverse library is formed;

(c) selecting and isolating from said diverse library expression vector capable of producing V_H or V_L polypeptides which in combination have said catalytic activity;

(d) transforming a host cell with said expression vectors; and

(e) isolating a V_H and V_L polypeptide encoded by said vector from said host cell.

27. The V_H and V_L polypeptide of claim 26 wherein said amplified V_H and V_L coding sequences are joined into separate expression vectors.

28. The V_H and V_L polypeptides of any of claims 22-27 wherein said V_H and V_L polypeptides comprise an Fab.

29. The V_H and V_L polypeptides of any claims 22-27 wherein said V_H and V_L polypeptides comprise a whole antibody.

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Screening combinatorial antibody libraries for catalytic acyl transfer reactions

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Abstract. A bacteriophage λ vector system for the expression of Fab fragments from the mouse antibody repertoire in *Escherichia coli* has been described. We have used this system to generate a catalytic antibody from a combinatorial antibody library. Monoclonal antibody 43C9 was raised against a transition state analogue of the hydrolysis of carboxyamide. mRNA from hybridoma cells expressing this antibody was cloned into phage λ and clones that expressed the mRNA for either the heavy or the light chain of the antibody were isolated. These individual libraries were then crossed to generate a combinatorial library in which clones coexpressed the heavy and light chains. This library was screened for antibodies/Fab fragments that bound to the original antigen with high affinity. DNA sequencing showed that these fragments were the same as those in antibody 43C9. Three different clones were found to catalyse the hydrolysis of carboxyamide. More efficient expression vectors and improved screening techniques should lead to the isolation of many more catalytic antibodies from combinatorial antibody libraries.

1991 Catalytic antibodies. Wiley, Chichester (Ciba Foundation Symposium 159) p 145–155

Monoclonal antibodies are used extensively in various fields of biology and medicine. Some important applications include the investigation of cellular mechanisms, the isolation of interferons, cancer research, clinical diagnosis and gene product analysis. The generation of monoclonal antibodies with specific catalytic functions is an emerging technology that combines the high specificities of antibodies with chemical catalysis. A number of reactions have been successfully catalysed by monoclonal antibodies (for review see Lerner & Benkovic 1988). The production of homogeneous antibodies for catalysis is entirely dependent on the hybridoma technology; but this is an inefficient method

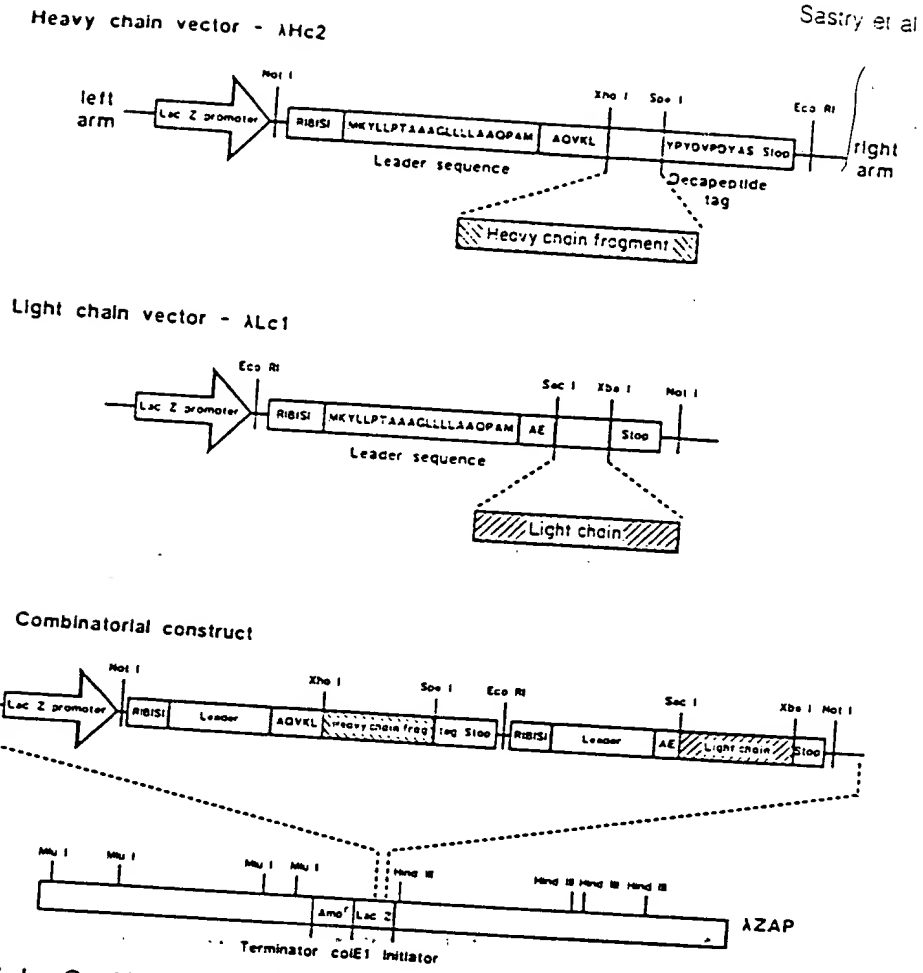

FIG. 1. Combinatorial bacteriophage λ vector system for expression of Fab antibody fragments. The LC1 vector is for cloning PCR products of mRNAs that code for κ light chains; the HC2 vector is for cloning PCR products of mRNAs coding for heavy chain Fd sequences. The combinatorial constructs that can express Fab fragments are generated by cutting light and heavy chain DNA at the antisymmetric *Eco*R1 site of each vector, followed by religation of the resulting arms.

for surveying the immunological repertoire and limits the number of catalysts that can be obtained. We have developed a system using bacteriophage λ to clone and express a combinatorial library of Fab fragments of the mouse antibody repertoire in *Escherichia coli* (Fig. 1) (Sastry et al 1989, Huse et al 1989). The system allows rapid and easy identification of monoclonal Fab fragments in a form suitable for genetic manipulation. However, it remains to be shown that such combinatorial libraries can be used to produce catalytic Fab

fr: .ents. In this paper we demonstrate the generation of a catalytic antibody from a combinatorial antibody library.

Using the λ phage system we generated an Fab combinatorial library from the spleen of a mouse immunized with phosphonamidate 1 (NPN), a transition state analogue for the hydrolysis of carboxyamide substrate 2 (Fig. 2). Screening the library with the antigen, NPN, linked to bovine serum albumin (NPN-BSA) resulted in the identification of a number of Fab fragments that bound to the antigen in a competitive manner. To find efficient catalysts for the hydrolysis of the nitroanilide 2, we screened the Fab combinatorial library directly for catalysis. The induced phage libraries were incubated with nitrocellulose filters saturated with the substrate, or the substrate was added directly to agar containing cells infected with the phage before they were poured onto a plate. Unfortunately, these approaches were unsuccessful because of the chemical nature of the reaction as well as the limited amount of Fab that is secreted by the phage molecules. It has previously been observed that catalysis of hydrolysis of the amide 2 occurs at 37 °C with high concentrations of an antibody (Janda et al 1988).

The high concentrations of antibody required for catalysis are difficult to achieve directly on the phage surface. Also, the product of the hydrolysis, *p*-nitroaniline, is diffusible and is hard to observe either directly on phage

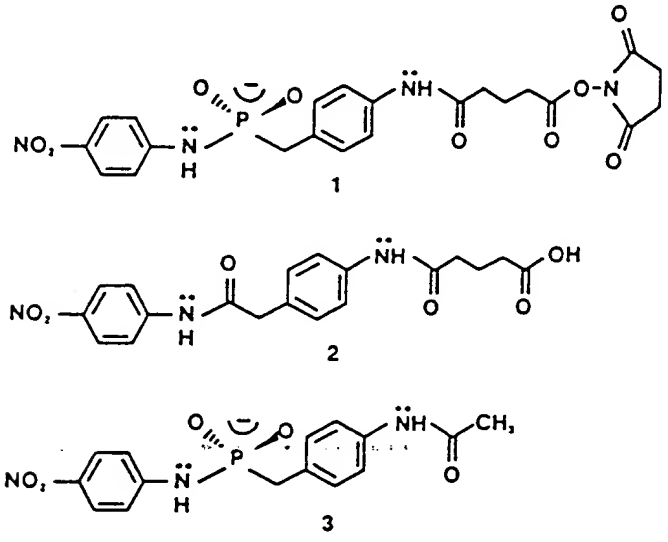

FIG. 2. The transition state analogue phosphonamidate 1 (NPN) which was used to induce antibodies that hydrolyse the carboxyamide substrate 2. The phosphonamidate functionality mimics the stereoelectronic features of the transition state for hydrolysis of the amide bond. The transition state analogue 3 is an inhibitor of the reaction.

plaques or on nitrocellulose filters. Because of these practical limitations, we decided to screen initially for Fab fragments that bound to NPN and then for those that showed catalytic activity. As an essential first step, we cloned and expressed a monoclonal antibody (43C9) that catalyses the amide hydrolysis in the phage system (Janda et al 1988). Besides being an internal control, the expression of the monoclonal antibody in phage also allows the study of its structure and mechanism of catalysis. Mutagenesis and chain-exchange experiments can be easily performed on the cloned antibody to improve its catalytic activity.

Methods

Total RNA from 10^7 43C9 hybridoma cells was isolated as described (Chomczynski & Sacchi 1987). The mRNAs were purified on an oligo dT column, then amplified using the polymerase chain reaction to obtain separate pools of heavy and light chain DNA (Sastry et al 1989, Huse et al 1989). Amplification of heavy chain DNA was performed with eight different 5′ primers and a 3′ primer specific for the IgG2b isotype. Light chain DNA was similarly amplified with five 5′ primers and a κ chain-specific 3′ primer. Heavy and light chain libraries were generated in phage λ and crossed to obtain an Fab combinatorial library (Huse et al 1989). This library was then screened with NPN-BSA labelled with ^{125}I and Fab fragments that bound the antigen were identified (Huse et al 1989). These Fab fragments were excised using helper phage (M12 mp8) and McBlue cells and plated on LB/ampicillin plates (Short et al 1988). Colonies on the plates represented the excised plasmid carrying the cloned heavy and light chain pieces.

Individual clones were grown up and their protein products isolated using an affinity column made from anti-$(Fab')_2$ coupled to Sepharose beads. Purified Fab was dialysed for 4–6 hours against ATE (Aces, Tris, ethanolamine) buffer, pH 9.0, concentrated to 1–3 μM solution, and used for catalysis. Catalysis was performed at 37 °C in ATE buffer at pH 9.0 with the 1–3 μM Fab solution and a saturating amount (1 mM) of substrate 2. Sequencing of the positive clones was as described by Sanger et al (1977).

Results

PCR amplification of heavy and light chain DNA resulted in bands of about 700 bp as analysed by agarose gel electrophoresis. A number of different primers were used for amplification from the hybridoma cells, because these may contain other non-functional heavy or light chains and restricted amplification may result in the cloning and expression of the wrong chains. To avoid this problem, we pooled the amplified DNA from the heavy and the light chains, then cloned each pooled fraction into the expression vector. Cloning of heavy chains resulted in 2×10^6 recombinants; the light chain library contained 5×10^5 recombinants.

ening of the heavy chain recombinants with an antibody raised against a conserved 10 amino acid sequence in the heavy chain showed that 90% of these were expressing the decapeptide and therefore the heavy chain. Anti-κ antibody screening of the light chain library indicated that 60% of the clones were expressing κ light chains. The combinatorial library consisting of 2×10^7 recombinants was screened with the anti-decapeptide and anti-κ antibodies; 65% of the clones coexpressed heavy and light chains.

The Fab library (3000 plaques/plate) was then screened with iodinated NPN-BSA and positive clones were identified after a three-day exposure. Fragments that bound the antigen (binders) were identified at a frequency of 1/200; this relatively low frequency may be due to the presence of non-functional heavy and light chains in the Fab library. Ideally, amplification of the hybridoma RNA with specific 5′ heavy and light chains should generate Fab fragments that bind at a much higher frequency.

The DNA sequences of the binders were obtained to identify the clone that exactly represents the monoclonal catalytic antibody 43C9. Comparison of the light chain deduced N-terminal amino acid sequence of antibody 43C9 and the deduced amino acid sequences of ten of the binders indicated that five of the clones (8a11, 8a12, 8a1, 7a2, 7a4) had the correct light chain. Three of these clones (8a1, 8a11, 8a12) were identical and differed from each of the other two (7a2, 7a4) by a single amino acid in the framework region. All the clones had the same heavy chain sequence; comparison with the N-terminal sequence of the authentic antibody was not possible because its N-terminus is blocked.

Purified Fab from each of the ten clones described above was assayed for catalytic activity; 8a11, 7a2 and 7a1 hydrolysed amide 2 at a rate clearly above the background rate (Fig. 3). The reaction was inhibited completely by the addition of transition state analogue 3, 20 μM. This indicated that the observed catalysis was exclusively due to the Fab. SDS-PAGE of the catalytic recombinant Fabs showed a single species at 50 kDa. Reducing conditions gave a doublet at 25 kDa, indicative of a single pure Fab fragment. Because of the limited amount of Fab produced in our system, detailed kinetic analysis has not been possible. Overexpression of the catalytic Fab is currently being sought, to facilitate the kinetic studies.

Discussion

The bacteriophage λ vector system developed for the expression of Fab fragments is ideally suited for studying the structure and mechanism of any desired monoclonal antibody. We have successfully expressed a monoclonal catalytic Fab in this system and have shown that it retains the ability to catalyse a specific amide hydrolytic reaction.

Future studies will be aimed at identifying more binders from the library which also display catalytic activity. The success of these will hinge upon our ability

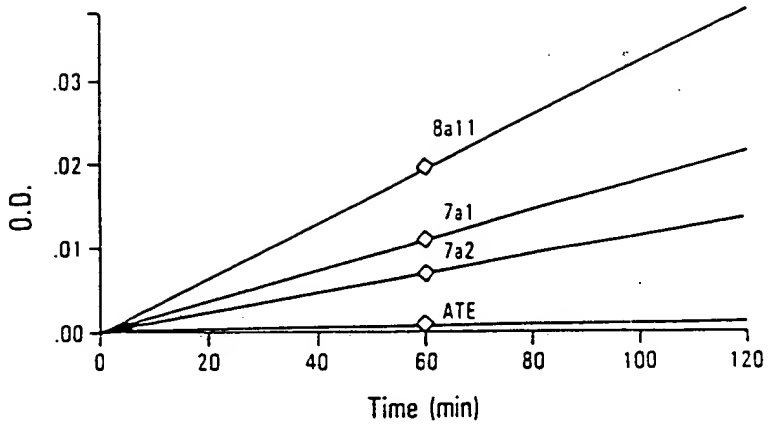

FIG. 3. Hydrolysis of carboxyamide 2 by Fab clones 8a11, 7a2 and 7a1. Hydrolysis was carried out at 37 °C with 2 μM antibody, 1 mM substrate in ATE buffer, pH 9.0. The differences in the observed rates seen for each clone probably reflect inaccuracies in protein concentration determination rather than clone differences. The background hydrolysis was measured with the substrate alone; in all cases the reaction was monitored at 405 nm.

to obtain a better system for expressing the protein, possibly utilizing Summer's baculovirus system (Smith et al 1983). More efficient screening for catalytic antibodies might be achieved via a genetic selection process.

Finally, a general solution to the antibody catalysis of a peptide bond may be obtained using the phage technology presented. Recently, we have constructed a single chain antibody with a coordination site for metals (Iverson et al 1990). When this site is incorporated into the light chain of an Fab fragment, a bound metal ion could act as a hydrolytic cofactor when properly aligned with a heavy chain which binds a small peptide sequence. The possibility of such a reaction appears remote; however, by taking advantage of the large numbers and combinations available through the combinatorial library, opportunities for success are within reach.

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DISCUSSION

Hansen: The exact placing of an amino acid is critical for enzyme catalysis. Do you have a sense, perhaps from Sargeson's work (Buckingham et al 1970), of how precise one has to be in orienting a carbonyl group near the metal ion to see catalysis?

Lerner: I don't know. They were basically looking at an intramolecular situation, because the substrate was directly bound to an open site on the coordination complex.

Martin: Another structural detail to consider is the geometry of the antibody's metal-binding ligands. A slight difference in the relative positions of metal-binding amino acid side chains could have a dramatic effect on the catalytic efficiency of the antibody. The coordination geometry of metals in natural enzymes is often distorted: for example, the tetrahedral geometry of cobalt-substituted carboxypeptidase A is markedly irregular compared to simple tetrahedral complexes of cobalt such as cobalt tetrachloride. In their entatic state hypothesis, Vallee & Williams (1968) proposed that the distorted coordination geometry is a critical feature of metalloenzymes in that it causes the metal to be unusually reactive—in their terms 'poised for catalysis'.

Lerner: Isn't that flying in the face of results from a great number of coordination complex experiments?

Jencks: Ground state strain of that kind can change the properties of the ions and the ligands and the metal, certainly; but to say that the ground state strain or distortion directly influences the transition state is wrong. It may provide a system which has a proper pK or oxidation potential or whatever, that will lead to a transition state more readily, and this might be done better with another metal that has a different size and a different potential, but it doesn't relate directly to the stability of the transition state.

Martin: If the enzyme or antibody binds a metal with tetrahedral geometry, say by three amino acid side chains and a reactive water molecule, the effect of the distorted coordination geometry might be to fine tune the pK of the metal-bound water molecule, thereby making it more reactive.